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Effect of discharged pulp and paper mill effluent on the blood morphology of *Heteropneustes fossilis* (Singhi)

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ABSTRACT

The present study was undertaken to know the effect of common mixed pulp and paper mill effluent discharge released from century pulp and paper mill Lalkuan, Nainital Uttarakhand on the blood morphology of *Heteropneustes fossilis* (Singhi). The various morphological changes were observed in the specimen, randomly collected with the help of cast net from (3 sites) different locations. The maximum changes were observed in the specimen collected from site 3 in compare to site 1 and 2 respectively. The formation of inclusion bodies, sickle cells, Cabot rings and ovalocytes were recorded along with nuclear fusion, membrane disruption and cell clumping of blood cells in the specimen collected from the site 3 *(i.e.* nearer to source of discharge) while in the specimen collected from site 2, the formation of tear drop cell, target cells, spherocytes and bi-nucleated cells were recorded along with the activity of cell clumping. The changes were found less in the specimen collected from site 1 in compare to the specimen of site 2 and 3 *i.e.* formation of ovalocytes, spherocytes and microcytes with the activity of membrane degeneration and agglutination in blood cells.

KEYWORDS

Agglutination, *Heteropneustes fossilis*, Inclusion Bodies, Pulp and Paper Mill Effluent

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The addition of unwanted discharges/ substances into the natural water ways/bodies cause changes in the physical, chemical and biological characteristics of the aquatic system which lead to ecological disturbances. The speedy industrialization in India has resulted a substantial increase in the liquid waste (spent wash or effluent) which is traditionally discharged on the open land or into nearby natural water, causing a number of environmental problems including threat to plants and animal lives present in that environment.

The industrial effluents contribute a lot to water pollution forming a threat to aquatic plants and animals (Ramona *et al*., 2001). These industrial pollutants alter the natural condition of aquatic medium that causes behavioural changes as well as morphological imbalance in aquatic organisms (Yadav *et al*., 2007). The pollution leads to a steady decline in the population of aquatic flora and fauna, particularly fishes. The Pollutants generally produce relatively quick changes in hematological characteristics of fish (Johansen *et al.,* 1994,

Rizkalla *et al.,* 1999). Hematology is used as an index of fish health status in the number of fish species to detect physiological changes following different stress conditions like exposure to pollutants, diseases, metals, hypoxia, etc. (Blaxhall, 1973, Duthie and Tort, 1985). Therefore, hematological techniques are the most common method to determine the sub-lethal effects of the pollutants (Larsson *et al*., 1985). Blood of any organism shows the direct relation between its health and in his surviving environment. There are three types of cellular elements presents in the blood *i.e.* RBCs, WBCs and platelets each of these cells have its own functions and differs morphologically from the others. To maintain the healthy state, the destruction and production of cells is balanced and therefore the number of cells present in the blood at any particular time is relatively constant.

The present work is based on the morphological studies of RBCs (erythrocytes). Erythrocytes are the most abundant cell type found in the peripheral blood and function in respiration by transporting oxygen to and carbon dioxide from body tissues (Michael and Stoskope, 1992). The main functions of RBCs are to carry Hb, which transports the oxygen to the tissues. The metabolic activities of RBCs also are capable of maintaining the Hb molecules in functional states. In normal morphological and cytological condition the RBCs have its own nucleus is extremely condensed. Erythrocytes morphology changes only in special external and internal conditions *i.e.* due to anemia and other pollutants of surrounding medium and in disease conditions.

Materials and Methods

Collection of Specimen

Waste water from the Century pulp and paper mill Lalkuan (Nainital) is drained into a small rivulet called Gola Nala which after about 20 km distance it merge into the Gola River. The specimens of *H. fossilis* were collected randomly from the above said source through cast net from 3 sites in different locations.

Morphological Study of Blood

Blood samples were collected on the well neat and clean slides from the caudal vein of the fishes by cutting the peduncle.

After blood collection, blood smears were made immediately. The slides were air dried for 1 hr. at room temperature and then fixed in 95% methanol at 4^0C (Michael and Stoskope, 1992). After that slides were stained with dyes following suitable standard methods and a cover slip was placed on top using glycerol. Slides were examined under electronic microscope attached with camera.

Results and Discussion

Normal erythrocytes in fishes have an oval shape structure with a rounded to oval central nucleus with densely packed chromatin (fig 1). The present study shows erythrocytic morphological alterations in the blood cells of *H. fossilis* exposed in the discharge of whole pulp and paper mill effluent (fig 2 and 4). A high percentage of red blood cells were in the process of losing their normal outline and cytoplasm, according to the peripheral blood smear examination at higher exposure levels. The result collaborate the finding of (Akinrotimi *et al.,* 2013) in Tilapia treated with Industrial Effluents. The various morphological changes were observed in the specimen, randomly collected with the help of cast net from (3 sites) different locations.

Mishra and Poddar (2013) observed almost same variation in the blood cells of freshwater Murrel (*Channa punctatus* Bloch), exposed to Phenolic industrial wastes of the Bhilai Steel Plant, Chhattisgarh, India. The maximum changes were observed in the specimen collected from site 3 in compared to site 1 and 2 respectively. The formation of inclusion bodies, sickle cells, Cabot rings and ovalocytes were recorded along with nuclear fusion, membrane disruption and cell clumping of blood cells in the specimens collected from the site 3, (*i.e.* nearer to source of discharge) while in the specimen collected from site 2, the formation of tear drop cell, target cells, spherocytes and bi-nucleated cells were recorded along with the activity of cell clumping. The changes were found less in the specimen collected from site 1 in compared to the specimen of site 2 and 3 *i.e.* formation of ovalocytes, spherocytes and microcytes with the activity of membrane degeneration and agglutination in blood cells. The almost similar results were also reported by (Misra *et al.*, 2016) in fresh water fish *Cyprinus carpio* exposed with different concentrations of pulp and paper mill effluent.

The various morphological changes in the blood cells of specimen like formation of tear drop cells indicates towards anoxic condition which may be due to low formation of RBCs.

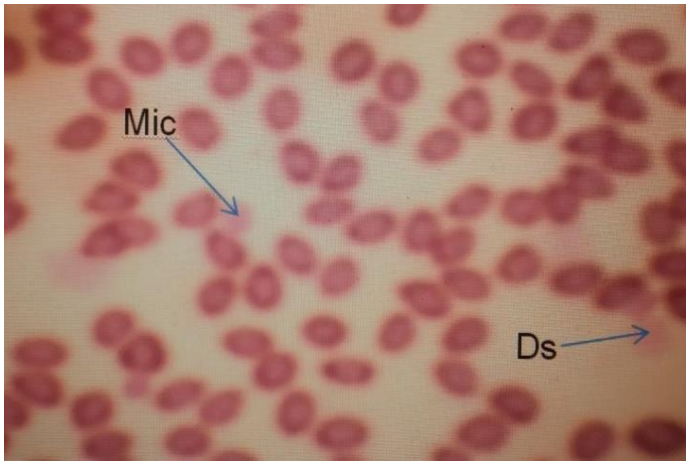

Fig 1. Control Group (Specimen from culture pond)

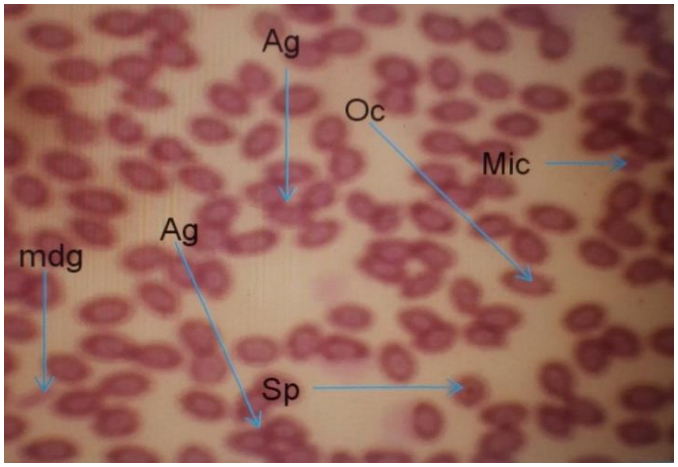

Fig 2. Site-1 (at Shantipuri, Pantnagar, Uttrakhand)

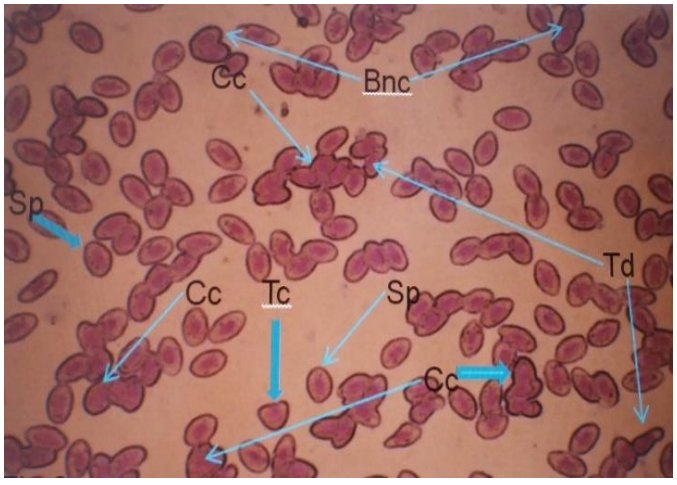

Fig 3. Site-2 towards (source of discharge) upstream from 1st site

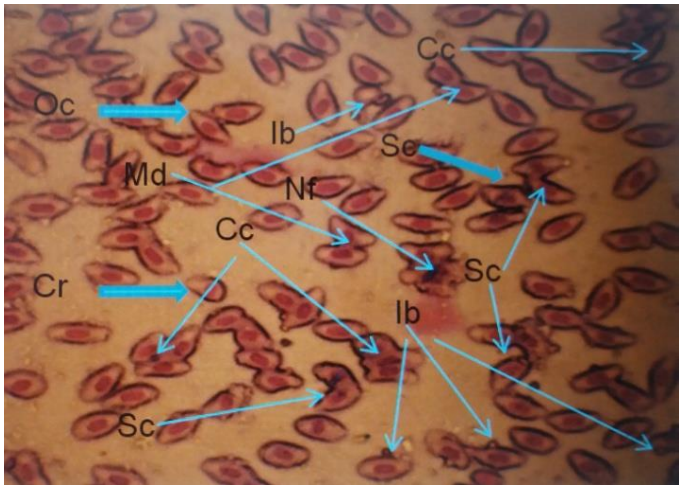

Fig 4. Site-3 towards upstream (nearer to source of discharge)

Table 1. Hematological abbreviations

S. N.	Terminology	Full Form
1	Ac	Accanthocytes
2	Ag	Agglutination
3 (10)	Agh	Hemoglobin agglutination
4 (3)	Bnc	Binucleated cells
5	Cc	Cell clumping
6	Cd	Cell diffusion
4	Cr	Cabot rings
8	Dmr	Donnanens membrane Ruptured
7	Ds	Discocytes
9	Ep	Elliptocytes
11	Hc	Hemoglobin Crystals
12	Hgn	Hemoglobin network
13	Hpc	Hypochromic RBCs
15	Ib	Inclusion bodies
14	Mac	Macrocyte
17	Md	Membrane dissruption
16	Mdg	Membrane degeneration
18	Mic	Microcyte
19	Nf	Nuclear fusion
20	Oc	Ovalocytes
21	Rf	Rouleaux formation
22	Sc	Sickle cells
23	Sp	Spherocytes
24	St	Stomatocytes
25	Tc	Target cell
26	Td	Tear drop cells

The sickle cell and membrane disruption directly indicates about low intensity of heavy metals toxicity in the specimen. The sickle cell, membrane diffusion, cabot rings and ovalocytes formation indicates towards metal toxicity and anemic condition as well as deteriorate water conditions (Yadav *et al.*, 2007).

The cell diffusion, agglutination, membrane degeneration, and cabot rings are main symptoms that indicates the pernicious anemia and lead poisoning. The formation of inclusion bodies along with the activity of nuclear fusion were also observed which, may be due to presence of heavy metal in the surrounding habitat of specimen (Larson *et al.*, 1985).

Conclusion

The present work has envisage that toxic substance present in the effluent leads to various detrimental effect on flora and fauna in natural water bodies, and ultimately affects the human life through bio-magnification and bio-accumulation via food chain. This research work needs further authentication.

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